



DECLARATION UNDER 37 C.F.R. §1.131 Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450	Application Number	09/919,643
	Attorney Docket Number	10991398-1
	Filing Date	July 31, 2001
	First Named Inventor	Diane D. Ilsley
	Examiner	Sue Xu Liu
	Group Art	1639
	Title	Methods for Depositing Small Volumes of Protein Fluids Onto the Surface of a Substrate

This Declaration and the attached Exhibit are being submitted in conjunction with the Applicants' Response to the Office Action dated February 27, 2007.

We, Diane Ilsley, Douglas Amorese, Michael Caren and Peter Tsang, do hereby declare as follows.

1. We are the inventors of the invention claimed in the above captioned application.
2. We have been asked to declare and provide factual evidence in support of conception of the above captioned application before April 24, 2001.
3. As evidenced by Exhibit A, we conceived of using an inkjet printhead to deposit protein reagents, e.g., antibodies, prior to April 24, 2001. The dates have been redacted from Exhibit A. However, a redacted date set forth in Exhibit A shows conception of the invention prior to April 24, 2001.
4. Exhibit A consists of a photocopy of an Invention Disclosure (total of 9 pages) in which details of methods for depositing a quantity of fluid containing a protein of interest, e.g., an antibody, onto the surface of a substrate are described.

5. Pages 1 to 2 of Exhibit A are internal Invention Disclosure forms used by the Legal Department of Agilent Technologies. Pages 3 to 9 of Exhibit A, describe the details of the methods of the invention. In brief, methods for depositing a quantity of fluid containing a protein of interest, e.g., an antibody, onto the surface of a substrate is set forth. This invention allows for the deposition of a protein onto the surface of a substrate without substantially modulating the activity or functionality of the protein.
6. We hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Respectfully submitted,

Date: _____

Diane Ilsley

Date: _____

Douglas Amorese

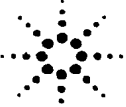
Date: _____

Michael Caren

Date: _____

Peter Tsang

Attachments: Exhibit A



Agilent Technologies



INVENTION DISCLOSURE

PAGE ONE OF

PDNO 10003540 DATE RCVD

ATTORNEY GMS/BSP

Instructions: The information contained in this document is COMPANY CONFIDENTIAL and may not be disclosed to others without prior authorization. Submit this disclosure to the Agilent Technologies Legal Department as soon as possible. No patent protection is possible until a patent application is authorized, prepared, and submitted to the Government.

Descriptive Title of Invention:

Use of thermal inkjets for deposition of proteins and multiple reagents in a reaction

Name of Project:

Product Name or Number:

Was a description of the invention published, or are you planning to publish? If so, the date(s) and publication(s): NO

Was a product including the invention announced, offered for sale, sold, or is such activity proposed? If so, the date(s) and location(s): NO

Was the invention disclosed to anyone outside of AGILENT TECHNOLOGIES, or will such disclosure occur? If so, the date(s) and name(s): NO

If any of the above situations will occur within 3 months, call your IP attorney or the Legal Department now at 1-553-3061 or 408-553-3061.

Was the invention described in a lab book or other record? If so, please identify (lab book #, etc.):

DC notebook # 2024, pg 43-44, 50-55 PT notebook # 2735, pg 133-137

Was the invention built or tested? If so, the date:

Tested

Was this invention made under a government contract? If so, the agency and contract number: NO

Description of Invention: Please preserve all records of the invention and attach additional pages for the following. Each additional page should be signed and dated by the inventor(s) and witness(es).

- A. Prior solutions and their disadvantages (if available, attach copies of product literature, technical articles, patents, etc.).
- B. Problems solved by the invention.
- C. Advantages of the invention over what has been done before.
- D. Description of the construction and operation of the invention (include appropriate schematic, block, & timing diagrams; drawings; samples; graphs; flowcharts; computer listings; test results; etc.).

Signature of Inventor(s): Pursuant to my (our) employment agreement, I (we) submit this disclosure on this date: []

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(If more than four inventors, include additional information on another copy of this form and attach to this document)

S1**INVENTION DISCLOSURE**

COMPANY CONFIDENTIAL

PAGE 2 OF 2

Signature of Witness(es): (Please try to obtain the signature of the person(s) to whom invention was first disclosed.)

The invention was first explained to, and understood by, me (us) on this date: []

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Signature

Date of Signature

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Use of thermal inkjets for deposition of proteins and multiple reagents in a reaction

Prior solutions:

Bioanalytical microsystems, such as biosensors and assays in a microarray format or reaction chamber, often require the use of a technology that can dispense very small quantities (pico- and nanoliters) of solutions containing biomolecules. The technology must be rapid, highly reproducible, and deposit solutions with precise placement onto a given solid support. Several recent papers have described the design and construction of devices for the production of microarrays of biomolecules onto solid support (1-4).

In particular, a method is described using a conventional inkjet printer for the microdeposition of proteins (3). The black ink was removed from an HP ink cartridge and the cartridge was extensively washed with water. The cartridge was filled with the protein deposition solution using a microsyringe and sealed. The protein solution was then deposited onto a solid support and allowed to air dry, where it remained active for 1-2 weeks when stored at 4°C. One problem with this method is a minimum of 2 mL of solution was needed to fill the cartridge. Unused sample could be recovered, with the exception of 200-300 μ L that remained in the print head. Other problems include only a single solution can be loaded at time and loading is done manually. Similarly, Deeg et al. describe a method using bubble-jet technology for the metered application of an analytical liquid to a target. Their method is based on the manufacture of disposable jet units containing the analytical liquid in prepacked form. A preloaded jet may be cost effective, but lacks flexibility. Several different solutions can be delivered by aligning several jet elements in a row, rather than prepacking several different solutions into a single inkjet.

Description of the invention:

This invention uses an inkjet for deposition of extremely small quantities of reagents directly in a reaction chamber or on a solid support. The reagents are front loaded into the inkjet head. Multiple reagents can be loaded simultaneously and the amount of material needed is 2 μ L. The reagents are then spotted onto the solid support or reaction chamber. The inkjet can then be washed and reloaded with a different set of reagents.

Demonstration of feasibility:

Using Biodot 3 and a Hobbes inkjet head, 2 μ L of 100 μ g/mL bovine albumin serum (BSA) in 50 mM Tris-HCl, pH 7.5, 50 mM NaCl, and 0.05% SDS was loaded into 6 reservoirs of the inkjet. The concentration of the BSA solution was determined using Bradford Reagent. The head was fired multiple times, and the solution was collected for analysis (6 μ L). Two microliters of the pre- and post-fired solutions were analyzed using the Caliper prototype protein LabChip assay. Figure 1 shows these results. The overlaid electropherograms show that the BSA was not degraded during the firing of the inkjet and that the protein concentration of the pre- and post-fired solutions are relatively equal. The concentration of the first load of BSA into the inkjet is slightly

lower than the pre-fired and the second loaded BSA solution, indicating that some protein does bind irreversibly in the head the first time protein is loaded.

The second experiment shows that post-fired proteins are functional. A mouse monoclonal antibody to dsDNA (500 ug/mL total protein) was loaded into 6 reservoirs of the inkjet, fired multiple times, and collected (10 uL). Two microliters were analyzed using the Caliper prototype LabChip assay. Figure 2 shows that the pre- and post-fired protein spectrums are identical. Functionality of the antibody was determined by binding to dsDNA. The pre- and post-fired antibody solutions (8 uL) were incubated in 250 uL NETG buffer (150 mM NaCl, 5 mM EDTA, 48 mM Tris-HCl, pH 7.4, 0.25% gelatin) on a cDNA microarray at room temperature for 1 hour. The slide was then washed once with 1x NETG and 3 times with 1/100x NETG. A secondary antibody conjugated to R6G (goat anti-mouse-R6G, 1/50 dilution in 250 uL NETG) was added and incubated for 1 hour at room temperature to detect binding of the primary antibody to dsDNA. The slide was washed as described above, and scanned on the Axon scanner. Figures 3 and 4 show that the pre- and post-fired antibody solutions gave similar fluorescent signals and specificity for binding the DNA. Firing the antibody through the inkjet does not appear to affect functionality.

Experiments in progress:

1. Washing experiments to determine if proteins can be removed from the inkjet. A solution of 5 proteins (M_r =116,000, 97,000, 66,000, 45,000, and 18,500) of known concentration will be loaded into the head, fired, and collected. The head will be washed, and loaded with buffer. The buffer will be fired and collected. The concentration of the pre- and post-fired protein and buffer solutions will be determined using the Caliper protein LabChip assay.
2. Deposition of multiple reagents on a surface for a reaction. A glass slide containing inkjet deposited cDNAs that are crosslinked to the surface will be used. Cy5-dCTP will be hand-spotted randomly onto the surface and allowed to dry. A solution containing buffer and dNTPs and second solution containing DNA polymerase will be loaded into the inkjet and fired onto the glass slide. The slide will be incubated in a humid chamber at 37C for 60 minutes to allow DNA polymerization. The slide will be washed to remove unincorporated Cy5-dCTP. The slide will then be scanned for covalently linked Cy5-dCMP to the DNA attached to the surface, indicating that the DNA polymerase synthesized DNA.

Problems solved and advantages of the invention:

The described invention has the following advantages:

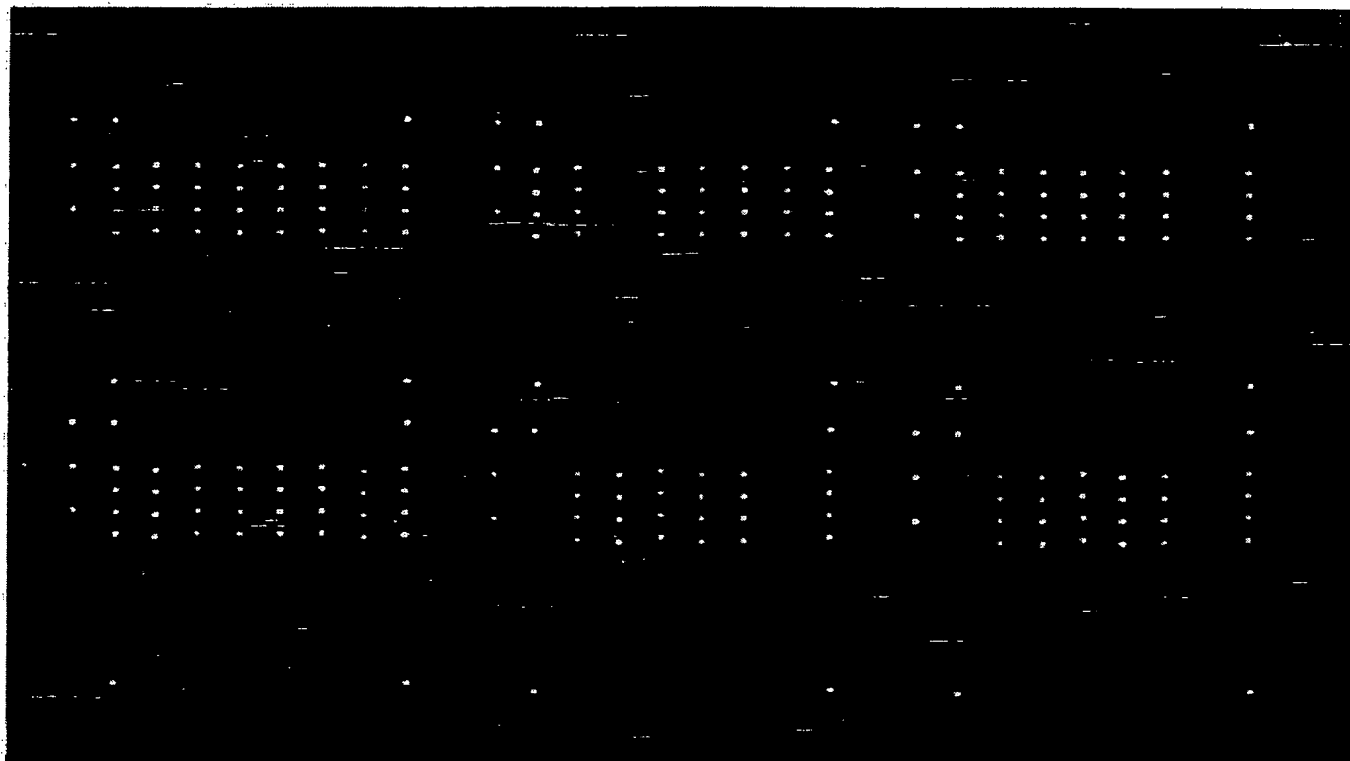
1. Only 2 uL of each solution is needed for loading into the inkjet, significantly reducing the reagent cost.
2. Multiple different solutions can be loaded simultaneously. The number of different solutions loaded is dependent upon the type of inkjet. The multi reservoir head allows for individual components to be delivered from individual reservoirs and different reaction mixtures can be composed during the printing process.

3. The inkjet can be washed and reloaded with a different set of reagents.
4. The process can take place in a temperature and humidity controlled environment.

1. Graves, D.J., Su, H.J., McKenzie, S.E., Surrey, S., and Fortina, P. (1998) Anal. Chem. 70, 5085-5092.
2. Yershov, G., Barsky, V., Belgovskiy, A., Kirillov, E., Kreindlin, E., Ivanov, L., Parivno, S., Guschin, D. (1996) Proc. Natl. Acad. Sci. USA 93, 4913-4918.
3. Roda, A., Guardigli, M., Russo, C., Pasini, P., and Baraldini, M. (2000) Biotechniques 28, 492-496.
4. Deeg et al., (Aug. 16, 1994) US patent #5,338,688.

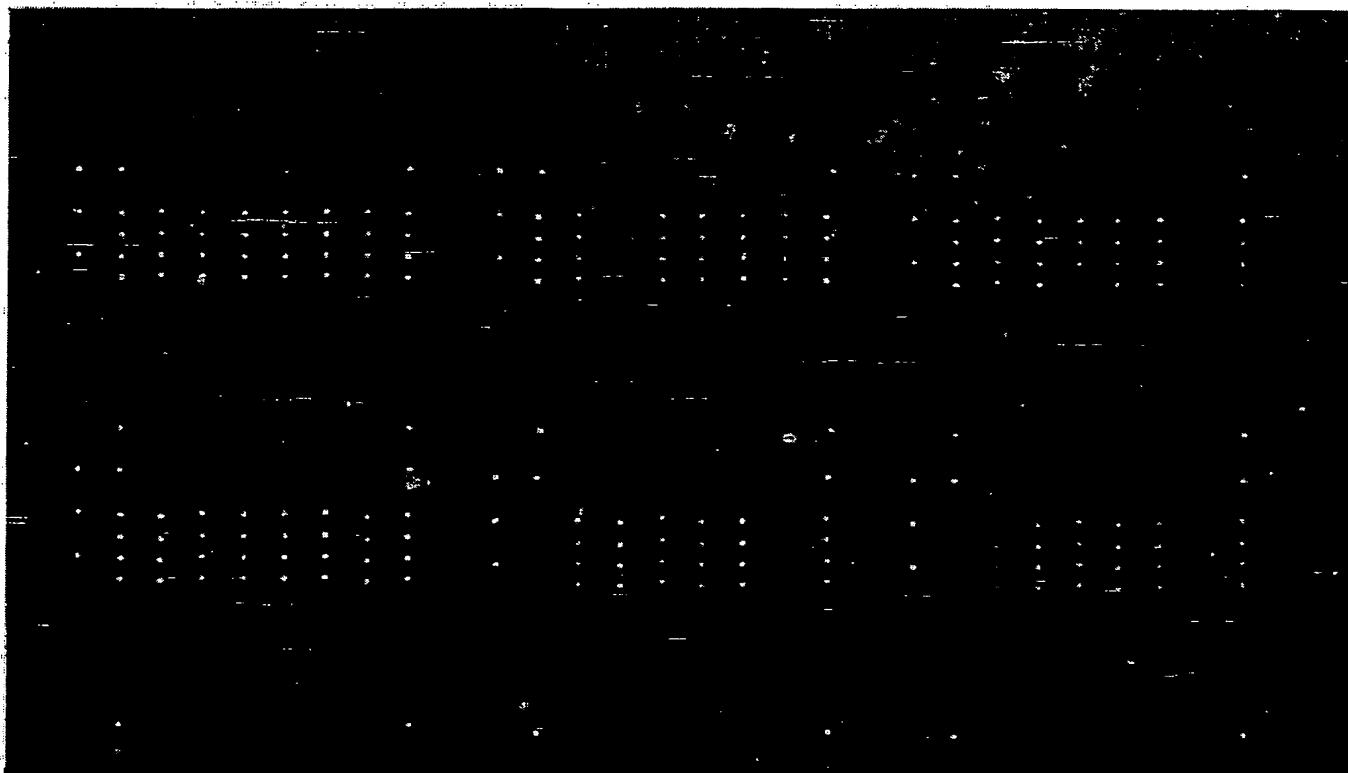
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Green color range: 0 to 1500



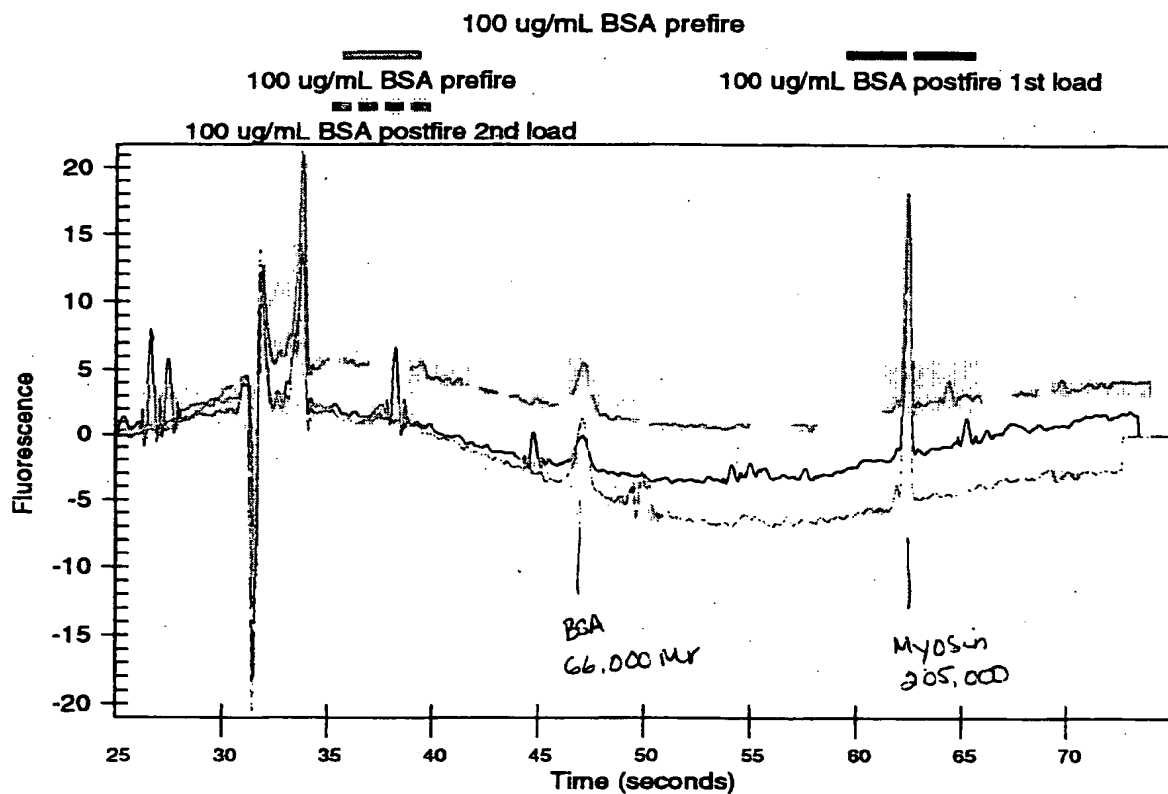
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Assay: Alpha Protein

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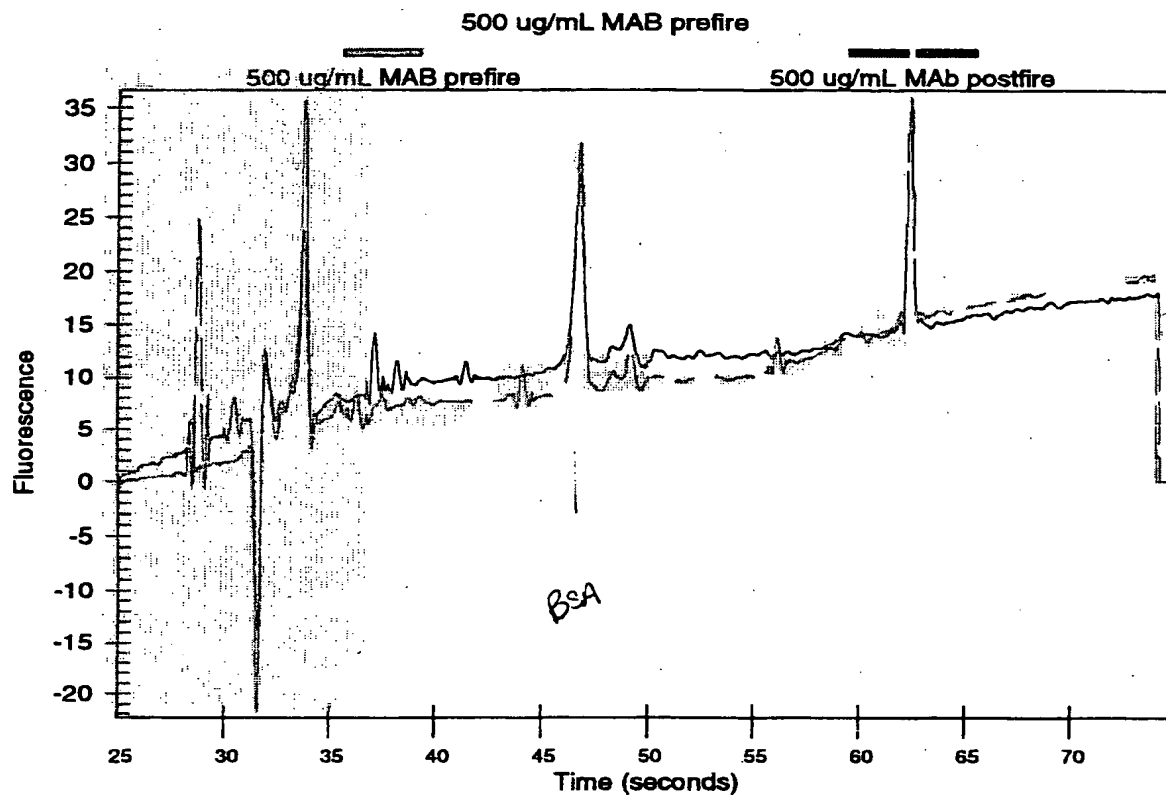


Peak	Mid. Time(sec)	Corr. Area	Size(KDa)	Conc.(ng/ul)
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Figure 1

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 Assay: Alpha Protein

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Peak	Mid. Time(secs)	Corr. Area	Size(KDa)	Conc.(ng/ul)
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Figure 2